Block Copolymers

Field of the Invention

The present invention is concerned with a class of block copolymers and the production therefrom of physiologically soluble polymer therapeutics, functionalised polymers, pharmaceutical compositions and materials.

Background of the Invention

Polymer Therapeutics are developed for biomedical applications requiring physiologically soluble polymers and include biologically active polymers, polymer-drug conjugates, polymer-protein conjugates, and other covalent constructs of polymer with bioactive molecules. An exemplary class of a polymer-drug conjugate is derived from copolymers of hydroxypropyl methacrylamide (HPMA) which have been extensively studied for the conjugation of cytotoxic drugs for cancer chemotherapy. An HPMA copolymer conjugated to doxorubicin, known as PK-1, is currently in Phase II evaluation in the UK. PK-1 displayed reduced toxicity compared to free doxorubicin in the Phase I studies. The maximum tolerated dose of PK-1 was 320 mg/m^2 which is 4-5 times higher than the usual clinical dose of free doxorubicin.

The polymers used to develop Polymer Therapeutics may also be separately developed for other biomedical applications where the polymer conjugate is developed (e.g. as a block copolymer) to form aggregates such as polymeric micelles and complexes. The polymers used to develop Polymer Therapeutics may also be separately developed for other biomedical applications that require the polymer be used as a material rather than as a physiologically soluble molecule. Thus, drug release matrices (including microspheres and nanoparticles), hydrogels (including injectable gels and viscious solutions) and hybrid systems (e.g. liposomes with conjugated poly(ethylene glycol) (PEG) on the outer surface) and devices (including rods, pellets, capsules, films, gels) can be fabricated for tissue or site specific drug delivery. Polymers are also clinically widely used as excipients in drug formulation. Within these three broad application areas: (1) physiologically soluble molecules, (2) materials and (3) excipients, biomedical polymers provide

a broad technology platform for optimising the efficacy of a therapeutic bioactive agent.

Therapeutic bioactive agents which can be covalently conjugated to a polymer include a drug, peptide and protein. Such conjugation to a soluble, biocompatible polymer can result in improved efficacy of the therapeutic agent. Compared to the free, unconjugated bioactive agent, therapeutic polymeric conjugates can exhibit this improvement in efficacy for the following main reasons: (1) altered biodistribution, (2) prolonged circulation, (3) release of the bioactive in the proteolytic and acidic environment of the secondary lysosome after cellular uptake of the conjugate by pinocytosis and (4) more favourable physicochemical properties imparted to the drug due to the characteristics of large molecules (e.g. increased drug solubility in biological fluids).

Co-block copolymers, comprising hydrophilic and hydrophobic blocks, form polymeric micelles in solution [Kataoka, Kwon, Yokoyama, Okano and Sakurai *J. Cont.Rel.* **1993**, *24*, 119,Gros, Ringsdorf and Schupp *Angew.Chemie Int. Ed. Eng.* **1981**, *20*, 301,Kwon, Yokoyama, Okano, Sakurai and Kataoka *Pharm. Res.* **1993**, *10*, 970,Kwon and Kataoka *Adv. Drug.Del.Rev.* **1995**, *16*, 295,Kwon and Okano *Adv. Drug Del. Rev.* **1996**, *21*, 107,Yokoyama *Crit.Rev.Therap.Drug Carrier Systems* **1992**, *9*, 213] and self-assembling micellar delivery systems are receiving increasing attention [Alakhov and Kabanov *Exp. Opin. Invest. Drugs* **1998**, *7*, 1453,Calibresi and Chabner *The Pharmacological Basis of Therapeutics* **1996**, 1225,Kabanov and Alakhov *J. Cont.Rel.* **1994**, *28*, 15,Yokayama, Okano, Sakurai and Kataoka *J. Cont. Rel.* **1994**, *32*, 269]. A significant advantage of these systems is the ability to design higher molecular weight micellar aggregates that will display prolonged circulation times that can maximise tumour capture by the EPR effect. Upon micelle disassociation, the individual block copolymer molecules are safely excreted, and as long as they are of low enough molecular weight these polymers can be non-biodegradable. For example, poly(ethylene glycol-aspartate) block copolymer doxorubicin conjugates form micelles ranging in size from 20-60 nm that accumulate in solid tumours and exhibit antitumour activity [Kataoka, Kwon, Yokoyama, Okano and Sakurai *J. Cont.Rel.* **1993**, *24*, 119,Kwon, Yokoyama, Okano, Sakurai and Kataoka *Pharm. Res.* **1993**, *10*,

970,Kwon and Kataoka *Adv. Drug.Del.Rev.* **1995**, *16*, 295,Kataoka *Controlled drug delivery - challenges and strategies* **1997**, 49,Yokoyama, Okano, Sakurai, Ekimoto, Shibazak and Kataoka *Cancer Res.* **1991**, *51*, 3229]. The doxorubicin is conjugated by its free amine directly to either the a- or b- pendent carboxylates in the poly(aspartic acid) block. Frequently physical entrapment of drug has accompanied conjugation [Yokoyama, Fukushima, Uehara, Okamoto, Kataoka, Sakurai and Okano *J. Cont. Rel.* **1998**, *50*, 79] and with stable block copolymer micelles, drug entrapment has become a viable strategy to deliver cytotoxic drugs to tumours [Alakhov and Kabanov *Exp. Opin. Invest. Drugs* **1998**, *7*, 1453,Yokoyama, Fukushima, Uehara, Okamoto, Kataoka, Sakurai and Okano *J. Cont. Rel.* **1998**, *50*, 79,Batrakova, Dorodnych, Klinskii, Kliushnenkova, Shemchukova, Goncharova, Ajakov, Alakhov and Kabanov *Br. J. Cancer* **1996**, *74*, 1545,Venne, Li, Mandeville, Kabanov and Alakhov *Cancer Res.* **1996**, *56*, 3626,Inoue, Chen, Nakamae and Hoffman *J. Cont. Rel.* **1998**, *51*, 221].

Poly(acrylic acid), poly(methaacrylic acid) and poly(ethylene glycol) based excipients are widely used to modify adhesion, swelling and pH dependent properties of tablets and pharmaceutical formulations. Incremental variation in the stoichiometry of the conjugation reactions of functionalised amines provide libraries of narrow MWD candidate polymers. This will make it possible to optimise the materials properties that include thermal properties, crystallisation, adhesion, swelling, coating and pH dependent conformation either independently or collectively. Of these many materials properties, controlling the rate of crystallisation processes tends to influence the stability, solubility and activity of chemically and biologically sensitive drugs (e.g. proteins). Hence, functionalised excipients designed to slow crystallisation processes and maintain unstable amorphous morphologies of pharmaceutical formulations (i.e. blends) may find wide use.

Additionally copolymeric excipients [Kabanov, Alakov and Batrakova *PCT-WO 99/39731* **1999**, 80 pages,Galakatos, Langer and Putnam *PCT-WO059627* **2000**, 46 pages,Alakhov, Klinski, Li, Piertrzynski, Venne, Batrakova, Bronitch and Kabanov *Colloids Surf., B.* **1999**, *16*, 114,Lemieux, Guerin, Paradis, Proulz, Chistyakova, Kabanov and Alakhov *Gene Ther.* **2000**, *7*, 986] and nanoscopic

particles [Boal, Ilhan, DeRouchey, Thurn-Albrecht, Russell and Rotello *Nature* **2000**, *404*, 746] have been examined. Many excipients that are generally recognised as safe have been evaluated to determine a multitude of trends that can be matched to the physicochemical properties of the pharmcologically active compounds. A doxorubicin formulation using a combination of two pluronics has shown this formulation may have broader efficacy than current clinical formulations of doxorubicin [Alakhov and Kabanov *Exp. Opin. Invest. Drugs* **1998**, *7*, 1453]. Since coblock polymers form aggregated micellar structures these may be potentially developed into novel formulations for the oral administration of bioactive agents.

Polymer-drug conjugates tend to be non-uniform with respect to molecular weight of the polymer and the location and number of conjugating pendent chains along the polymer mainchain. Polymer therapeutics must be rigorously characterised with respect to their molecular weight and polydispersity since biodistribution and pharmacological activity are known to be molecular weight-dependent. For example, blood circulation half-life [Cartlidge, Duncan, Lloyd, Kopeckova-Rejmanova and Kopecek *J Con. Rel.* **1986**, *4*, 253], renal clearance, deposition in organs [Sprincl, Exner, Sterba and Kopecek *J. Biomed. Mater. Res.* **1976**, *10*, 953], rates of endocytic uptake [Duncan, Pratten, Cable, Ringsdorf and Lloyd *Biochem. J.* **1981**, *196*, 49,Cartlidge, Duncan, Lloyd, Rejmanova and Kopecek *J. Cont. Rel.* **1986**, *3*, 55] and biological activity can depend on polymer molecular weight characteristics [Kaplan *Anionic Polymeric Drugs* **1980**, 227,Ottenbrite, Regelson, Kaplan, Carchman, Morahan and Munson *Polymeric Drugs* **1978**, 263,Butler *Anionic Polymeric Drugs* **1980**, 49,Muck, Rolly and Burg *Makromol. Chem.* **1977**, *178*, 2773,Muck, Christ and Keller *Makromol. Chem.* **1977**, *178*, 2785,Seymour *J. Bioact. Compat. Polymers* **1991**, *6*, 178]. While HPMA copolymers currently undergoing clinical evaluation exhibit increased efficacy and a considerable amount of the biological rationale for polymer-drug conjugates has been elucidated, the fact is these therapeutic compounds exist as broad statistical distributions in respect to molecular weight and structure of conjugation pendent chains. This is problematic from a regulatory standpoint, especially for chronic conditions. For example, it would be difficult to ascertain if long term effects

were due to low or high molecular weight species in a polydisperse therapeutic conjugate.

Currently many candidate copolymer-drug conjugates are prepared by a reaction on a polymer or a polymer analogous reaction of a low molecular weight drug and an active ester polymeric precursor with a small number of reactive repeat units [Kopecek and Bazilova *Eur. Polymer J.* **1973**, *9*, 7,Strohalm and Kopecek *Angew. Makromol. Chem.* **1978**, *70*, 109,Rejmanova, Labsky and Kopecek *Makromol. Chem.* **1977**, *178*, 2159,Kopecek *Makromol. Chem.* **1977**, *178*, 2169,Rihova, Ulbrich, Strohalm, Vetvicka, Bilej, Duncan and Kopecek *Biomaterials* **1989**, *10*, 335,Kopecek *J. Cont. Rel.* **1990**, *11*, 279].

Many conjugates have been prepared by the polymer analogous reaction however the competitive hydrolysis of the *p*-nitrophenol ester actually produces conjugates that have pendent chains terminated with either drug, carboxylate, or aminopropanol [Configliacchi, Razzano, Rizzo and Vigevani *J. Pharm. Biomed. Analysis* 1996, *15*, 123,Pinciroli, Rizzo, Angelucci, Tato and Vigevani *Magn. Reson. Chem.* 1997, *35*, 2]. The free radical precipitation polymerisation gives the active ester polymeric precursor with a small number of reactive repeat units as a random copolymer typically with a polydisersity ranging from 1.3-2.5 and above depending on the pendent chain. Also such precipitation polymerisation strategies only give polymeric precursors with a small range of molecular weights. Incorporation of different amounts monomers with different pendent chains requires that the polymerisation conditions be optimised to obtain reproducible molecular weights under the renal threshold. In principal, it is possible to alter drug loading by varying its stoichiometry during conjugation but the final polymeric conjugate will contain mixtures of the unreacted pendent chains with out drug that are statistically distributed over a broad molecular weight distribution. Lysosomal degradation of non-drug conjugated pendent chains will compete with degradation of the drug conjugated pendent chains. This competition complicates the pharmacology and pharmacokinetics for polymer-drug conjugates. From the viewpoint of drug regulatory authorities, this strategy for preparing conjugates result in final polymer-drug conjugates that are extremely varied in structure and thus difficult to regulate as a medicinal agent.

In addition to regulatory issues and as mentioned above, structural heterogeneity will influence the pharmacology and pharmacokinetics of therapeutic conjugates. For example, the rate of drug release from a given polymer chain can vary according to the structure of the pendent chain and drug [Duncan *Anti-Cancer Drugs* **1992**, *3*, 175,Duncan, Seymour, Ulbrich, Spreafico, Grandi, Ripamonti, Farao and Suarato *Eur. J. Cancer* **1991**, *27*, S52]. Rates of release are also influenced by the amount (i.e. loading) and location along the polymer mainchain of the conjugated drug. As greater amounts of hydrophobic drug are conjugated onto a soluble hydrophilic polymer, the possibility increases to form unimolecular polymeric micelles which may hinder access of the lysosomal enzymes to degrade the linker and release the conjugated drug [Ulbrich, Konak, Tuzar and Kopecek *Makromol. Chem.* **1987**, *188*, 1261]. Hydrophobic drugs conjugated to hydrophilic polymers can result in a lower critical solution temperature (LCST) where phase separation occurs and the conjugate becomes insoluble. Also, as a drug is released from a polymer-drug conjugate, it would be expected that changes in polymer conformation will occur that might lead to differences in drug release rate with time [Pitt, Wertheim, Wang and Shah *Macromol. Symp.* **1997**, *123*, 225,Shah, Werthim, Wang and Pitt *J. Cont. Rel.* **1997**, *45*, 95] and therefore influence pharmacological properties. The extent and location of drug loading and its influence on polymer solution properties is an important, and yet poorly understood phenomenon that has a fundamental effect on the *in vivo* properties of therapeutic polymer-conjugates.

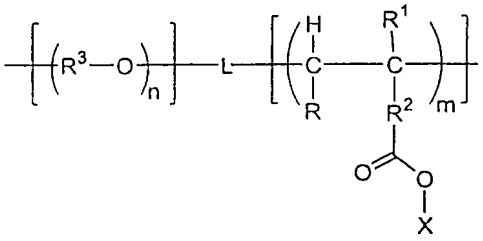
It is evident that as mixtures of structures, the polydisperse and randomly conjugated polymer-drug conjugates which are being studied are not optimal. While a significant amount is known about the biological rationale for the development of the polymer therapeutics, there is less known about the chemical rationale. Three broad needs related to chemical structure worthy of systematic study have been identified to extend the use of soluble addition polymers in medicine: (1) preparation of relevant polymers with narrow molecular weight and pendent chain distribution, (2) use of suitable conjugation strategies that minimise competitive reactions and (3) controlled placement of

conjugating pendent chains along the polymer mainchain (e.g. preparation of block copolymers).

To address these chemical limitations for preparing therapeutic conjugates, WO 01/18080 describes the production of low molecular weight distribution homo-and copolymers, including block copolymers, having a polydispersity less than 1.4. Polymerisation was carried out by controlled radical polymerisation processes to give narrow molecular weight polymeric precursors that are used as precursor polymers to prepare a wide range of metha- and acrylamide homo-and copolymers. Only a few metha- and acrylamide homo-and copolymers with narrow molecular distribution can be prepared directly from polymerization. These are used in the production of polymer drug conjugates having desirable biological profiles.

Summary of the Invention

In a first aspect of the present invention, there is provided a block copolymer comprising the unit (I)



(I)

wherein R is selected from the group consisting of hydrogen, C_1-C_{18} alkyl, C_2-C_{18} alkenyl, C_7-C_{18} aralkyl, C_7-C_{18} alkaryl, C_6-C_{18} aryl, carboxylic acid, C_2-C_{18} alkoxycarbonyl, C_2-C_{18} alkaminocarbonyl, or any one of C_1-C_{18} alkyl, C_2-C_{18} alkenyl, C_7-C_{18} aralkyl, C_7-C_{18} alkaryl, C_6-C_{18} aryl, C_2-C_{18} alkoxycarbonyl and C_2-C_{18} alkaminocarbonyl substituted with a heteroatom within, or attached to, the carbon backbone; R^1 is selected from the group consisting of hydrogen and C_1-C_6 alkyl groups; R^2 is a linking group; X is an electron withdrawing group; R^3 is selected from the group consisting of C_1-C_{18} alkylene, C_2-C_{18} alkenylene, C_7-C_{18} aralkylene, C_7-C_{18} alkarylene and C_6-C_{18} arylene; L is a divalent linker joining the blocks; and m and n are each an integer of greater

than 1.

The copolymer (I) is an A-B type block copolymer. It may be an A-B-A or A-B-C type block copolymer. The substructures defined in the square parentheses are the blocks. Preferably, m and n are integers of 5 to 300, more preferably 10 to 200, most preferably 25 to 150.

Preferably the block copolymer has a polydispersity of less than 1.4, preferably less than 1.2 and a molecular weight (Mw) of less than 100,000. Preferably (I) is water soluble.

X is preferably individually selected for each block and may be the same or different.

The electron withdrawing group X is preferably a carboxylate activating group, and is preferably selected from the group consisting of N-succinimidyl, pentachlorophenyl, pentafluorophenyl, para-nitrophenyl, dinitrophenyl, N-phthalimido, norbornyl, cyanomethyl, N-pyridyl, N-trichlorotriazine, 5-chloroquinilino, and N-imidazole. Preferably X is an N-succinimidyl or imidazole moiety.

Preferably R is selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_1-C_6 alkenyl, C_1-C_6 aralkyl and C_1-C_6 alkaryl, C_2-C_8 alkoxycarbonyl, C_2-C_8 alkaminocarbonyl. Most preferably R is selected from hydrogen and methyl.

Preferably R^1 is selected from the group consisting of hydrogen, methyl, ethyl, propyl, butyl, pentyl or isomers thereof. Most preferably R^1 is selected from hydrogen and methyl.

Preferably R^2 is selected from a bond or contains at least 1 carbon atom or at least 1 heteroatom.

Where R^2 is not a bond, R^2 is connected to CR^1 via a divalent group, preferably comprising a carbonyl, C_1-C_{18} alkylene and/or C_6-C_{18} arylene group which may be substituted with 1 or more heteroatoms. Preferably R^2 comprises a group selected from the group consisting of C_1-C_6 alkylene, C_6-C_{12} arylene, C_1-C_{12} oxyalkylene and carbonyl-C_1-C_6 alkylene. Where R^2 comprises an alkylene group, it can be branched, linear or cyclical, substituted or unsubstituted with one or more alkyl groups, and is preferably methylene, 1,2-ethylene, 1,3-propylene, hexylene or octylene. Where R^2

comprises an arylene group, preferably it is benzylene, tolylene or xylylene.

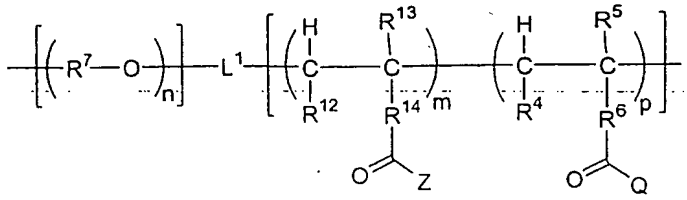
Preferably the groups R^3, which may be the same or different, are selected from the group consisting of C_1-C_8 alkylene groups, preferably 1,2-alkylene, and C_6-C_{12} arylene groups, most preferably methylene, ethylene, 1,2-propylene and 1,3-propylene. Preferably all groups R^3 are the same, most preferably all are 1,2-ethylene or 1,2-propylene.

L preferably comprises a C_1-C_{18} alkylene or C_6-C_{18} arylene group which may be substituted and/or interrupted with 1 or more heteroatoms. Preferably L comprises a group selected from the group consisting of C_1-C_6 alkylene, C_6-C_{12} arylene, C_1-C_{12} oxyalkylene and C_1-C_6 acyl. Where L comprises an alkylene group, it can be branched, linear or cyclical, substituted or unsubstituted with one or more alkyl groups, and is preferably methylene, 1,2-ethylene, 1,2-propylene, 1,3-propylene, ^{tert}butylene, ^{sec}butylene, hexylene or octylene. Where L comprises an arylene group, it is preferably benzylene, tolylene or xylylene. Most preferably L comprises a -COR^a group, wherein R^a is selected from the group consisting of C_1-C_6 alkylene or C_6-C_{12} arylene, preferably methylene, 1,2-ethylene, 1,2-propylene, 1,3-propylene, ^{tert}butylene and ^{sec}butylene.

The block copolymer of the present invention may incorporate other polymeric, oligomeric or monomeric blocks. For example, further polymeric blocks incorporated in the polymer may comprise acrylic polymers, alkylene polymers, urethane polymers, amide polymers, polypeptides, polysaccharides and ester polymers.

The molecular weight of the block copolymer should ideally be less than 100,000, preferably less than 50,000 where the block copolymer is to be used as a physiologically soluble block copolymer (in order that the renal threshold is not exceeded, ie to ensure that the polymer is cleared from the kidney glomerulus). Preferably the molecular weight of the block copolymer is in the range of 4000-50,000, more preferably 25,000-40,000.

A further preferred aspect of the present invention provides a block copolymer comprising the structure (II)



(II)

wherein R^4 is selected from the group consisting of hydrogen, C_1-C_{18} alkyl, C_2-C_{18} alkenyl, C_7-C_{18} aralkyl, C_7-C_{18} alkaryl, C_6-C_{18} aryl, carboxylic acid, C_2-C_{18} alkoxycarbonyl, C_2-C_{18} alkaminocarbonyl, or any one of C_1-C_{18} alkyl, C_2-C_{18} alkenyl, C_7-C_{18} aralkyl, C_7-C_{18} alkaryl, C_6-C_{18} aryl, C_2-C_{18} alkoxycarbonyl, and C_2-C_{18} alkaminocarbonyl substituted with a heteroatom within, or attached to, the carbon backbone; R^5 is selected from the group consisting of hydrogen and C_1-C_6 alkyl groups; R^6 is a linking group; Q is a solubilising group selected from the group consisting of hydroxyl, C_1-C_{12} alkyl, C_2-C_{12} alkenyl, C_7-C_{12} aralkyl, C_7-C_{12} alkaryl, C_1-C_{12} alkoxy, C_1-C_{12} hydroxyalkyl, C_1-C_{12} alkylamino, C_1-C_{12} hydroxyalkylamino, or any of C_1-C_{12} alkyl, C_2-C_{12} alkenyl, C_7-C_{12} aralkyl, C_7-C_{12} alkaryl, C_1-C_{12} alkoxy, C_1-C_{12} hydroxyalkyl, C_1-C_{12} alkylamino, C_1-C_{12} alkylamino substituted with an amine, hydroxyl, carbonyl or thiol group; R^7 is selected from the group consisting of C_1-C_{18} alkylene, C_2-C_{18} alkenylene, C_7-C_{18} aralkylene, C_7-C_{18} alkarylene and C_6-C_{18} arylene; n, m and p are each an integer of greater than 1; R^{12} is selected from the group consisting of hydrogen, C_1-C_{18} alkyl, C_2-C_{18} alkenyl, C_7-C_{18} aralkyl, C_7-C_{18} alkaryl, C_6-C_{18} aryl, carboxylic acid, C_2-C_{18} alkoxycarbonyl, C_2-C_{18} alkaminocarbonyl, or any one of C_1-C_{18} alkyl, C_2-C_{18} alkenyl, C_7-C_{18} aralkyl, C_7-C_{18} alkaryl, C_6-C_{18} aryl, C_2-C_{18} alkoxycarbonyl, and C_2-C_{18} alkaminocarbonyl substituted with a heteroatom within, or attached to, the carbon backbone; R^{13} is selected from the group consisting of hydrogen and C_1-C_6 alkyl groups; R^{14} is a linking group; L^1 is a divalent linker joining the blocks; Z is a pendent group selected from the group consisting of $OM_{1/d}^{d+}$, NR^8R^9, SR^{10}, OR^{11} and OX, wherein X is defined above, M is a metal ion and d is an integer of 1 or 2, R^8 comprises an alkyl group, preferably an aminoacyl substituted alkyl group, more preferably oligopeptidyl group; R^9 is selected from hydrogen, C_1-C_{18} alkyl, C_2-C_{18} alkenyl, C_7-C_{18} aralkyl, C_7-C_{18} alkaryl; R^{10} and R^{11} comprise a group which is individually selected from the

group consisting of hydrogen, C_1-C_{12} alkyl, C_2-C_{12} alkenyl, C_7-C_{12} aralkyl, C_7-C_{12} alkaryl and C_1-C_{12} hydroxyalkyl, and may contain one or more cleavable bonds and may comprise a bioactive agent.

M is preferably a sodium or potassium ion.

Z is preferably individually selected for each block and may be the same or different. Thus, different pendent groups may be attached to different blocks.

Q is preferably individually selected for each block and may be the same or different.

Preferably the block copolymer of this aspect of the invention has a polydispersity of less than 1.4, preferably less than 1.2 and a molecular weight (Mw) of less than 100,000. Preferably (II) is water soluble. The molecular weight of the block copolymer is preferably less than 50,000, more preferably in the range of 4000-50,000, most preferably 25,000-40,000.

Preferably R^4 is selected from the group consisting of hydrogen, C_1-C_6 alkyl, C_1-C_6 alkenyl, C_1-C_6 aralkyl and C_1-C_6 alkaryl, C_2-C_8 alkoxycarbonyl, C_2-C_8 alkaminocarbonyl. Most preferably R^4 is selected from hydrogen and methyl.

Preferably R^5 is selected from the group consisting of hydrogen, methyl, ethyl, propyl, butyl, pentyl or isomers thereof. Most preferably R^1 is selected from hydrogen and methyl.

Preferably R^6 is selected from a bond or contains at least 1 carbon atom or at least 1 heteroatom.

Where R^6 is not a bond, R^6 is connected to CR^5 via a divalent group, preferably comprising a carbonyl, C_1-C_{18} alkylene and/or C_6-C_{18} arylene group which may be substituted with 1 or more heteroatoms. More preferably R^6 comprises a group selected from the group consisting of C_1-C_6 alkylene, C_6-C_{12} arylene, C_1-C_{12} oxyalkylene and carbonyl-C_1-C_6 alkylene. Where R^6 comprises an alkylene group, it can be branched, linear or cyclical, substituted or unsubstituted with one or more alkyl groups, and is preferably methylene, 1,2-ethylene, 1,2-propylene 1,3-propylene, ^{tert}butylene, ^{sec}butylene, hexylene or octylene. Where R^6 comprises an arylene group, it is preferably benzylene, tolylene or xylylene.

Preferably the groups R^7, which may be the same or different, are selected from the group consisting of C_1-C_8 alkylene groups, preferably 1,2-alkylene, and C_6-C_{12} arylene groups, most preferably methylene, ethylene, 1,2-propylene and 1,3-propylene. Preferably all groups R^7 are the same, most preferably all are 1,2-ethylene or 1,2-propylene.

Z may comprise a protecting group, ie be a group OX, where X is defined above.

Z may comprise a peptidic group. Preferably Z comprises one or more aminoacyl groups, preferably 2-6 aminoacyl groups, most preferably 4 aminoacyl groups. In a particularly preferred embodiment group Z comprises a glycine-leucine-phenylalanine-glycine linker. The aminoacyl linker is most preferably a peptide linker capable of being cleaved by preselected cellular enzymes, for instance, those found in the liposomes found in cancerous cells.

In a further aspect, group Z comprises a cis-aconityl group. This group is designed to remain stable in plasma at neutral pH (~7.4), but degrade intracellularly by hydrolysis in the more acidic environment of the endosome or liposome (~pH 5.5-6.5). This is particularly advantageous for the treatment of cancer as there are marked improvements in therapeutic efficacy and site specific passive capture through the enhanced permeability and retention (EPR) effect. The EPR effect results from enhanced permeability of macromolecules or small particles within the tumour neovasculature due to leakiness of its discontinuous endothelium. In addition to the tumour angiogenesis (hypervasculature) and irregular and incompleteness of vascular networks, the attendant lack of lymphatic drainage promotes accumulation of macromolecules that extravasate. This effect is observed in many solid tumours for macromolecular agents and lipids. Thus, increased accumulation in such tumours leads to a targeted delivery of a group Z incorporating a bioactive agent (as discussed below) and a cis-aconityl group.

The pendent chain Z may additionally comprise a ligand or bioactive agent. The ligand may be any ligand which is capable of polyvalent interactions. Preferred bioactive agents are anti-cancer agents such as

doxorubicin, daunomycin and paclitaxel. The bioactive agent is preferably joined to $R^{14}CO$ via a peptidic linker.

L^1 preferably comprises a C_1-C_{18} alkylene and/or C_6-C_{18} arylene group which may be substituted and/or interrupted with 1 or more heteroatoms. Preferably L^1 comprises a group selected from the group consisting of C_1-C_6 alkylene, C_6-C_{12} arylene, C_1-C_{12} oxyalkylene and C_1-C_6 acyl. Where L^1 comprises an alkylene group, it can be branched, linear or cyclical, substituted or unsubstituted with one or more alkyl groups, and is preferably methylene, 1,2-ethylene, 1,2-propylene 1,3-propylene, ^{tert}butylene, ^{sec}butylene, hexylene or octylene. Where L^1 comprises an arylene group, preferably it is benzylene, tolylene or xylylene. Most preferably L^1 comprises a $-COR^a$ group, wherein R^a is defined above with regard to (I).

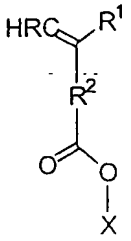
It should be understood that the graphical representation of (II) above is not intended to limit the order within the blocks bordered by parentheses, ie. the substructure containing Q may be adjacent to the block containing R^7.

Preferably, p is an integer of 1 to 500, more preferably 20 to 200.

Preferred definitions of R^{12}, R^{13} and R^{14} are the same groups as R^4, R^5 and R^6 respectively.

Preferably Q comprises an amine group attached to the R^6CO carbonyl carbon, preferably a C_1-C_{12} hydroxyalkylamino group, most preferably a 2-hydroxypropylamino group. This group is designed to be a solubilising group for the block copolymer in aqueous solutions. Generally the block copolymer of the present invention is a water soluble polyacrylamide/polyalkyleneglycol block copolymer, preferably a polymethacrylamide or polyethacrylamide/polyethyleneglycol block copolymer.

In a further aspect, the present invention provides a process for the production of a block copolymer, comprising the polymerisation of ethylenically unsaturated monomers including a compound (III)

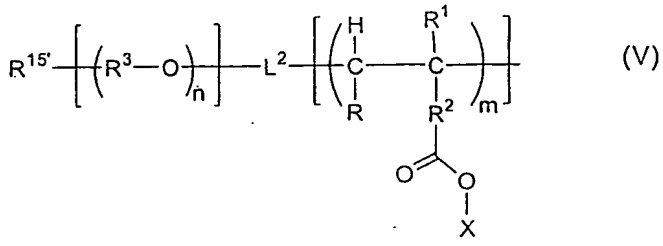


(III)

wherein R is selected from the group consisting of hydrogen, C_1-C_{18} alkyl, C_2-C_{18} alkenyl, C_7-C_{18} aralkyl, C_7-C_{18} alkaryl, C_6-C_{18} aryl, carboxylic acid, C_2-C_{18} alkoxycarbonyl, C_2-C_{18} alkaminocarbonyl, or any one of C_1-C_{18} alkyl, C_2-C_{18} alkenyl, C_7-C_{18} aralkyl, C_7-C_{18} alkaryl, C_6-C_{18} aryl, C_2-C_{18} alkoxycarbonyl, and C_2-C_{18} alkaminocarbonyl substituted with a heteroatom within, or attached to, the carbon backbone; R^1 is selected from the group consisting of hydrogen and C_1-C_6 alkyl groups; R^2 is a linking group; X is an electron withdrawing group; in the presence of an initiator compound (IV)

(IV)

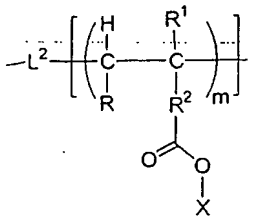
wherein n is an integer of 1 or more and Y is a radical initiating group; R^3 is selected from the group consisting of C_1-C_{18} alkylene, C_2-C_{18} alkenylene, C_7-C_{18} aralkylene, C_7-C_{18} alkarylene and C_6-C_{18} arylene; R^{15} comprises a group selected from the group consisting of hydrogen, C_1-C_{18} alkyl, C_2-C_{18} alkenyl, C_7-C_{18} aralkyl, C_7-C_{18} alkaryl and C_6-C_{18} aryl, C_1-C_{18} alkoxy, C_2-C_{18} alkeneyloxy, C_7-C_{18} aralkoxy, C_7-C_{18} alkaryloxy, C_6-C_{18} aryloxy and -O-Y; to produce a block copolymer comprising the unit (V)



(V)

wherein m and n are as defined above and L^2 is a divalent linking group

derived from Y and $R^{15'}$ is R^{15}, or where R^{15} is -O-Y, R^{15} is



Preferred examples of the definitions of X, R, R^1, R^2 and R^3 are as defined above in respect of (I).

Y preferably comprises a halogen substituted C_1-C_{18} alkyl or C_6-C_{18} aryl group, preferably bromine or chlorine substituted. Preferably Y comprises a group selected from the group consisting of C_1-C_6 alkyl, C_6-C_{12} aryl, C_1-C_{12} oxyalkyl and C_1-C_6 acyl substituted with 1 or more halogen atoms. Where Y comprises an alkyl group, it can be branched, linear or cyclical, substituted or unsubstituted with one or more alkyl groups, and is preferably methyl, ethyl, propyl, ^{tert}butyl, ^{sec}butyl, hexyl or octyl. Where Y comprises an aryl group, it is preferably benzyl, tolyl or xylyl. Most preferably Y comprises a -COR^y group, wherein R^y is selected from the group consisting of halogen substituted C_1-C_6 alkyl or C_6-C_{12} aryl, preferably methyl, ethyl, propyl,, ^{tert}butyl and ^{sec}butyl. Most preferably Y is -CO^{tert}butylbromide.

L^2 is preferably derived from Y, ie, the product of the radical reaction with monomer, and is a C_1-C_{18} alkylene and/or C_6-C_{18} arylene group which may be substituted with 1 or more heteroatoms. Preferably L^2 comprises a group selected from the group consisting of C_1-C_6 alkylene, C_6-C_{12} arylene, C_1-C_{12} oxyalkylene and carbonyl-C_1-C_6 alkylene. Where L^2 comprises an alkylene group, it can be branched, linear or cyclical, substituted or unsubstituted with one or more alkyl groups, and is preferably methylene, 1,2-ethylene, 1,2-propylene, 1,3-propylene, ^{tert}butylene, ^{sec}butylene, hexylene or octylene. Where L^2 is an arylene group, preferably it is benzylene, tolylene or xylylene. Most preferably L^2 is a -COR^a group, wherein R^a is selected from the group consisting of C_1-C_6 alkylene or C_6-C_{12} arylene, preferably methylene, 1,2-ethylene, 1,2-propylene, 1,3-propylene, ^{tert}butylene and ^{sec}butylene.

R^{15} is preferably selected from hydrogen, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_2-C_{10} alkenyl, C_7-C_{10} aralkyl, C_7-C_{10} alkaryl and C_6-C_{10} aryl and -O-Y, more preferably is methoxy or -O-Y (which will produce an A-B-A type block copolymer).

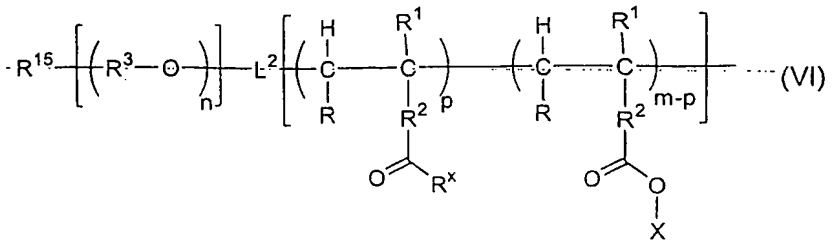
Preferably the process is a controlled radical polymerization.

Where the polymerization is carried out by atom transfer radical polymerization, the polymerisation is preferably carried out in the presence of a polymerisation mediator comprising a Cu(I) complex. Such complexes are usually Cu(I)Br complexes, complexed by a chelating ligand. Typical mediators are Cu(I)Br $(Bipy)_2$, Cu(I)Br (Bipy), Cu(I)Br (Pentamethyl diethylene), Cu(I)Br[methyl $_6$ tris(2-aminoethyl)amine] and Cu(I)Br(N, N, N', N'', N''-pentamethyldiethylenetriamine).

The ethylenically unsaturated monomers may include comonomers copolymerisable with the monomer of the formula (III).

The reaction should take place in the presence of a suitable solvent. Such solvents are generally aprotic solvents, for example tetrahydrofuran, acetonitrile, dimethylformamide, acetone, dimethylsulphoxide, ethyl acetate, methylformamide, ethylene carbonate and sulpholane and mixtures thereof. Alternatively, water may be used. Particularly preferred solvents are dimethylsulphoxide, ethylene carbonate, tetrahydrofuran, and dimethylformamide and mixtures thereof.

Preferably (V) may be reacted further with a reagent HR^x, wherein R^x is selected from the group consisting of $NR^{19}R^{20}$, SR^{21} and OR^{22}, wherein R^{19} is or comprises a linker group, preferably a substituted alkyl group, more preferably a peptidic group; R^{20} is selected from hydrogen, C_1-C_{18} alkyl, C_2-C_{18} alkenyl, C_7-C_{18} aralkyl, C_7-C_{18} alkaryl, C_6-C_{18} aryl; R^{21} and R^{22} are selected from the group consisting of hydrogen, C_1-C_{12} alkyl, C_1-C_{12} alkenyl, C_1-C_{12} aralkyl, C_1-C_{12} alkaryl, C_1-C_{12} alkoxy and C_1-C_{12} hydroxyalkyl, any of which may comprise a bioactive agent substituent and/or may contain one or more cleavable bonds, to form a derivatised block copolymer having the structure (VI)



(VI)

wherein $1 \leq p \leq m$.

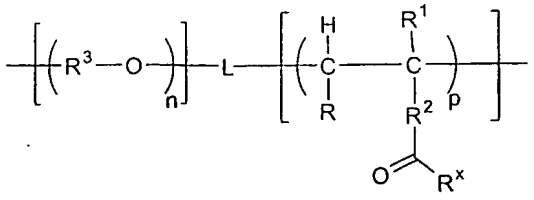
Preferably, p is an integer of 1 to 200, more preferably 1 to 10.

Preferably HR^x is H_2NR^z.

HR^x is generally a nucleophilic reagent capable of displacing X-O, to form a covalent bond with the acyl group attached to R^2. Most preferably R^z comprises a cleavable bond such as a aminoacyl linker or a cis-aconityl linker as described hereinbefore. Generally R^z comprises a bioactive agent substituent, which may have been attached prior to reaction with (V).

Subsequent to the production of a block copolymer having the structure (VI), an additional step of quenching the block copolymer may take place. This involves reacting the previously unreacted groups COOX with a quenching group. This group preferably comprises an amine moiety and is generally selected to be a solubilising or solubility modifying group for the block copolymer. Such a quenching compound is, for example a hydrophilic reagent, for example, hydroxypropylamine. Different types of quenching groups may be employed in the same polymer.

Alternatively (I) may be reacted with a reagent HR^x as defined above, to form a compound (VII)



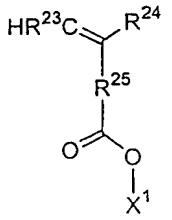
(VII)

wherein R, R^1, R^2, R^3, R^x, n, p and L are as defined above. This compound may be further reacted with a quenching group. These groups react with any

unreacted groups COOX. This group preferably comprises an amine moiety and is generally selected to be a solubilising or solubility modifying group for the block copolymer. Such a quenching compound is, for example a hydrophilic reagent, for example, hydroxypropylamine.

In a further aspect, the present invention provides a process for the production of a block copolymer, comprising the steps of

(1) polymerising ethylenically unsaturated monomers comprising a compound (VIII)



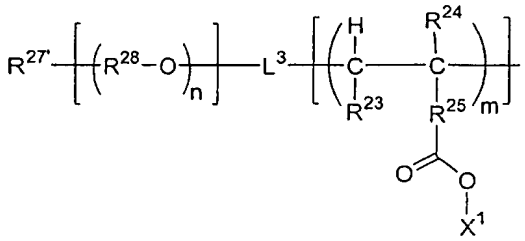
(VIII)

wherein R^{23} is selected from the group consisting of hydrogen, C_1-C_{18} alkyl, C_2-C_{18} alkenyl, C_7-C_{18} aralkyl, C_7-C_{18} alkaryl, C_6-C_{18} aryl, carboxylic acid, C_2-C_{18} alkoxycarbonyl, C_2-C_{18} alkaminocarbonyl, or any one of C_1-C_{18} alkyl, C_2-C_{18} alkenyl, C_7-C_{18} aralkyl, C_7-C_{18} alkaryl, C_6-C_{18} aryl, C_2-C_{18} alkoxycarbonyl, and C_2-C_{18} alkaminocarbonyl substituted with a heteroatom within, or attached to, the carbon backbone; R^{24} is selected from the group consisting of hydrogen and C_1-C_6 alkyl groups; R^{25} is a linking group; X^1 is selected from the group consisting of carboxyl activating groups, hydrogen, $M^1_{1/d}{}^{d+}$ and carboxyl protecting groups, wherein M^1 is a metal ion and d is an integer of 1 or 2; R^{26} is selected from the group consisting of C_1-C_{18} alkylene, C_2-C_{18} alkenylene, C_7-C_{18} aralkylene, C_7-C_{18} alkarylene and C_6-C_{18} arylene; in the presence of an initiator compound (VIII)

$$R^{27}(R^{28}O)_n—Y^1 \qquad \text{(IX)}$$

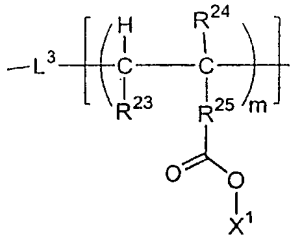
wherein n is an integer of 1 or more and Y^1 is a radical initiating group, R^{27} comprises a group selected from the group consisting of hydrogen, C_1-C_{18} alkyl, C_2-C_{18} alkenyl, C_7-C_{18} aralkyl, C_7-C_{18} alkaryl and C_6-C_{18} aryl; C_1-C_{18} alkoxy, C_2-C_{18} alkeneyloxy, C_7-C_{18} aralkoxy, C_7-C_{18} alkaryloxy, C_6-C_{18} aryloxy

and -O-Y^1; and R^{28} is selected from the group consisting of C_1-C_{18} alkylene, C_2-C_{18} alkenylene, C_7-C_{18} aralkylene, C_7-C_{18} alkarylene and C_6-C_{18} arylene; to produce a block copolymer comprising the unit (X)

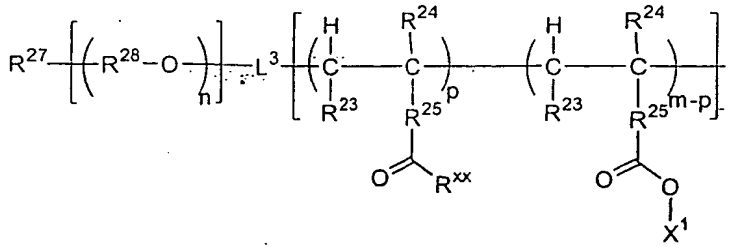


(X)

wherein m is an integer of greater than 1 and L^3 is a divalent linking group derived from L^3; and $R^{27'}$ is R^{27}, or where R^{27} is -O-Y^1, R^{27} is



(2) reacting (X) with a reagent HR^{xx}, wherein R^{xx} is selected from the group consisting of $NR^{29}R^{30}$, SR^{31} and OR^{32}, wherein R^{29} is a linker group, preferably a peptidic group; R^{30} is selected from hydrogen, C_1-C_{18} alkyl, C_2-C_{18} alkenyl, C_7-C_{18} aralkyl, C_7-C_{18} alkaryl, C_6-C_{18} aryl; R^{31} and R^{32} are individually selected from the group consisting of hydrogen, C_1-C_{12} alkyl, C_1-C_{12} alkenyl, C_1-C_{12} aralkyl, C_1-C_{12} alkaryl, C_1-C_{12} alkoxy and C_1-C_{12} hydroxyalkyl, and may contain one or more cleavable bonds, to form a derivatised block copolymer having the structure (XI)



(XI)

wherein $1 \leq p \leq m$.

Y^1 preferably comprises a halogen substituted C_1-C_{18} alkyl or C_6-C_{18} aryl group, preferably bromine or chlorine substituted. Preferably Y^1 comprises a group selected from the group consisting of C_1-C_6 alkyl, C_6-C_{12} aryl, C_1-C_{12} oxyalkyl and C_1-C_6 acyl substituted with 1 or more halogen atoms. Where Y^1 comprises an alkyl group, it can be branched, linear or cyclical, substituted or unsubstituted with one or more alkyl groups, and is preferably methyl, ethyl, propyl, ^{tert}butyl, ^{sec}butyl, hexyl or octyl. Where Y^1 comprises an aryl group, preferably it is benzyl, tolyl or xylyl. Most preferably Y^1 comprises -COR^y group, wherein R^y is defined above.

L^3 is preferably derived from Y^1 and is a C_1-C_{18} alkylene or C_6-C_{18} arylene group which may be substituted and/or interrupted with 1 or more heteroatoms. Preferably L^3 comprises a group selected from the group consisting of C_1-C_6 alkylene, C_6-C_{12} arylene, C_1-C_{12} oxyalkylene and carbonyl-C_1-C_6 alkylene. Where L^3 is an alkylene group, it can be branched, linear or cyclical, substituted or unsubstituted with one or more alkyl groups, and is preferably methylene, 1,2-ethylene, 1,2-propylene, 1,3-propylene, ^{tert}butylene, ^{sec}butylene, hexylene or octylene. Where L^3 is an arylene group, preferably it is benzylene, tolylene or xylylene. Most preferably L^3 comprises a -COR^a group, wherein R^a is selected from the group consisting of C_1-C_6 alkylene or C_6-C_{12} arylene, preferably methylene, 1,2-ethylene, 1,2-propylene, 1,3-propylene, ^{tert}butyl and ^{sec}butyl.

The electron withdrawing group X^1 is preferably a carboxylate activating group, and is preferably selected from the group consisting of N-succinimidyl, pentachlorophenyl, pentafluorophenyl, para-nitrophenyl, dinitrophenyl, N-phthalimido, norbornyl, cyanomethyl, N-pyridyl, N-trichlorotriazine, 5-chloroquinilino, and N-imidazole. Preferably X^1 is an N-succinimidyl or imidazole moiety.

R^{25} is preferably the same as R^2.

R^{27} is preferably selected from hydrogen, C_1-C_6 alkyl, C_1-C_6 alkoxy, C_2-C_{10} alkenyl, C_7-C_{10} aralkyl, C_7-C_{10} alkaryl and C_6-C_{10} aryl and -O-Y^1.

Preferably R^{28} is selected from the group consisting of C_1-C_8 alkylene groups and C_6-C_{12} arylene groups, most preferably methylene, ethylene,

propylene and isopropylene.

Preferably HR^{xx} is HR^{x} as defined above.

Preferably step (1) process is a controlled radical polymerization and (2) is a nucleophillic substitution reaction.

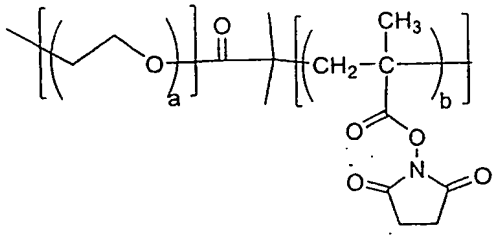
Detailed Description of the Invention

The present invention preferably provides a block copolymer having a polydispersity of less than 1.4, preferably less than 1.2. The block copolymer is preferably an activated polyacrylate ester that is prepared by Controlled Radical Polymerization. These block copolymers are designed to be derivitisable and may be used to form polymer-drug conjugates having improved biological profile.

The utility of the invention is that conjugation of a bioactive agent can be prepared in defined reagions of a polymer rather than randomly along the mainchain. The use of narrow molecular weight polymer precursor allows more efficient preclinical development to understand the range of aqueous solution based structure-property correlations that can be exploited to optimise the biological profile of polymer-drug conjugates.

The utilisation of the co-blocked polymeric precursors will allow for the preparation of water soluble, narrow MWD functionalised excipients that can be derived copolymers of poly(ethylene glycol) polyacrylic- and methacrylic acids that are further functionalised on the non-PEG block.

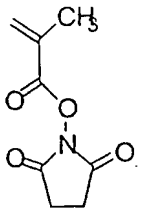
A particularly preferred block copolymer of the present invention comprises the structure (XII)



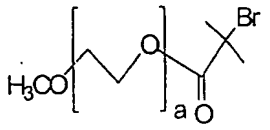
(XII)

wherein a and b are integers of 1 or more, and preferably define the blocks of the A-B type block copolymer. The activating moiety is an N-succinimidyl

group. This particular group has been found to be particularly stable in solution and resists spontaneous hydrolysis. This block copolymer may be produced by Atom Transfer Polymerization using a Cu(I)Br(pentamethyldiethylene) mediator. The polymerization involved the reaction of a monomer (XIII) with a polyethyleneglycol initiator compound (XIV) in a suitable aprotic solvent.



(XIII)



(XIV)

In one preferred embodiment the solvent is tetrahydrofuran. In another preferred embodiment the solvent is dimethylsulphoxide and optionally dimethylformamide in admixture thereof. A further particularly preferred embodiment uses ethylene carbonate as solvent. The reaction is preferably carried out under a nitrogen atmosphere and at a temperature of 0-150°C. A preferred temperature range is 30-80°C, most preferably 50-70°C.

The block copolymer comprising the unit (XII) may subsequently be derivatised. The carboxyl activating group may be substituted by a suitable nucleophilic reagent. In order to form polymer drug conjugates it is preferable to derivatise unit (XII) with a pendant moiety. Such a moiety could comprise a aminoacyl linker or a hydrolytically labile linker as defined hereinbefore. Such a linker can degrade when entering the lysosome of a diseased cell, thus releasing a drug or drug precursor directly to the target site.

Preferably a pendent moiety comprises a Gly-Leu-Phe-Gly linker or a

cis aconityl linker. Such a pendent linker may be covalently attached to a drug prior to block copolymer derivitisation or may be capable of being derivatised subsequent of attachment of the pendent moiety to the block copolymer backbone.

In a preferred embodiment the block copolymer comprising the unit (XII) is reacted with less than 1 equivalent of a pendent group, thus only substituting a pre-specified number of N-succinimidyl moieties. This allows a second, quenching step, which substitutes the remaining N-succinimidyl groups with a solubilising group. Such a group aids in the solubilisation of the block copolymer in aqueous solutions such as biological fluids. A preferred quenching agent should comprise a hydrophillic amine or amino acid, preferably a hydroxylated amine, for example 2-hydroxypropylamine. Amine terminated PEG may also be used. Alternatively, the carboxyl activating group may be hydrolysed to produce a free carboxylic acid moiety. An overview of a preferred reaction process is provided in scheme 1 below. In this particular example, the drug doxorubicin is attached to the block copolymer via a GLFG linker.

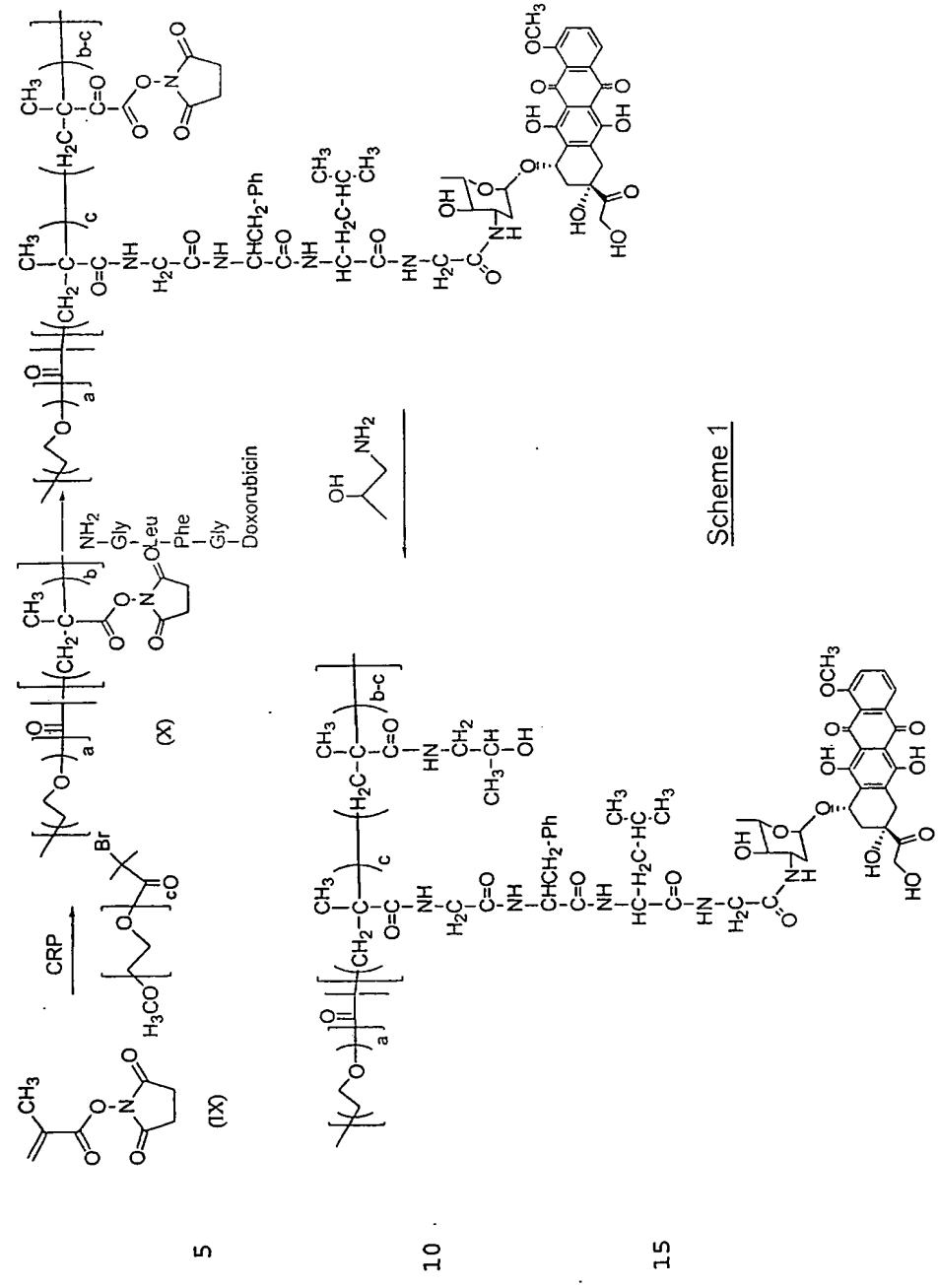
Preferably, a number of different bioactive agents may be conjugated to the polymer chain.

a and b are integers in the range of 1 to 500 and c is the number equivalent of pendent moieties reacted with the activated block copolymer. CRP processes are known to result in the presence of dormant initiating moieties at the chain ends of linear polymers.

The present invention is also concerned with the use of the block copolymers described above to prepare physiologically soluble polymer bioactive agent conjugates, polymer therapeutics, functionalised polymers, pharmaceutical compositions and materials.

Utilising the block precursor (XII) more controlled placement of the bioactive conjugating pendent chains along the polymer mainchain is possible because this conjugation will only occur in the block with the N-succinimidyl groups. This will result in co-block copolymers, comprising hydrophilic and hydrophobic blocks, to form polymeric micelles in solution. A significant advantage of these systems is the ability to design higher

molecular weight micellar aggregates that will display prolonged circulation times that can maximise tumour capture by the EPR effect. Upon micelle disassociation, the individual block copolymer molecules are safely excreted, and as long as they are of low enough molecular weight these polymers can be non-biodegradable. Additionally poly(acrylic acid), poly(methaacrylic acid) and poly(ethylene glycol) based excipients are widely used to modify adhesion, swelling and pH dependent properties of tablets and pharmaceutical formulations. The utilisation of the co-blocked polymeric precursor (XI) allows for the preparation of water soluble, narrow molecular weight distribution functionalised excipients derived copolymers of poly(ethylene glycol) polyacrylic- and methacrylic acids that are further functionalised on the non-PEG block. Since coblock polymers form aggregated micellar structures these new functionalised excipient may be potentially developed into novel formulations for the oral administration of bioactive agents.



Scheme 1

Example 1

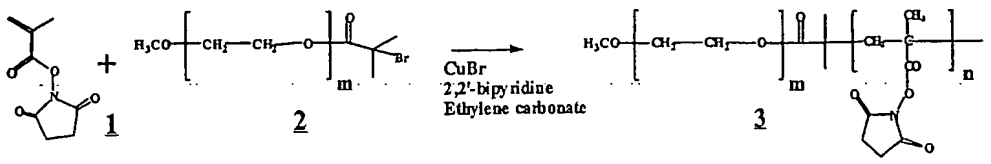
Preparation of macroinitiators 2.

The PEG (polyethylene glycol) macroinitiators were prepared by the procedure of Jankova et al (Macromolecules (1998), 31, 538-541). Triethylamine (12.5×10^{-3} mol, 1.265 g, 1.75 ml) in 15 ml dry CH_2Cl_2 was added to a 250 ml three-neck round-bottom flask equipped with a condenser, dropping funnel, gas inlet and a magnetic stirrer. After cooling to 0°C 2,2-bromoisobutyryl bromide (12.5×10^{-3} mol, 2,874 g, 1.55 ml) in 10 ml CH_2Cl_2 was added and the mixture purged with nitrogen. Then monomethoxy capped PEG (Mn = 2,000 g/mol) (5×10^{-3} mol, 10 g) in 50 ml CH_2Cl_2 was added dropwise during 1h under nitrogen. The PEG had been previously dried by azeotropic distillation in toluene and the residual toluene removed in vacuum.The temperature of the reaction mixture was allowed to rise to room temperature and the reaction continued for 18h. The solution was filtered, half of the solvent evaporated under vacuum and the product was precipitated in cold ether. The precipitate was recrystallised in absolute ethanol (stored overnight in the fridge). The macroinitiator was filtered, washed with cold ether and dried under vacuum. The crude product was purified by dissolving 4 g in 80 ml water. The solution pH was raised to pH 8 in order to hydrolyse the excess of i-BuBr. Then the solution was extracted with CH_2Cl_2 (70 ml). A stable emulsion was obtained and several hours were needed for complete phase separation. The solvent was removed in vacuum. The product was dissolved in hot EtOH and put in a fridge to crystallise. Then it was filtered and washed with ether and dried under vacuum. The purified product was white in colour. The degree of substitution calculated by the H MNR spectra.

This procedure was also used to prepare monomethoxy capped PEG macroinitiators derived from PEG 5000 and PEG 10000.

Example 2

Preparation of co-block, narrow molecular weight polymer precursors 3.



1 2 3

General polymerisation quantisations (reagents and conditions) are outlined in table 1

A mixture of monomer 1 (as synthesised in WO 01/18080), ethylene carbonate, and bipyridine was placed in a tube sealed with septum and it was purged with argon for 5 min and then the CuBr was added. The mixture was gently heated to form a solution (deep brown in colour) and purged with argon for another 30 min. Then a solution of the PEG macroinitiator 2 in the amount relative to the monomer specified in the table in ethylene carbonate (gently heated to melt both the ethylene carbonate and 2) was purged with argon for 10 min and added to the monomer solution by syringe washed with argon. The mixture was placed in a oil bath and stirred. The reaction was stopped by exposure to air, cooling and diluting with DMF. Then the solution was passed through a column filled with alumina and the polymer precipitated in MeOH. The precipitate was filtered, washed with ether and dried in vacuum. The product was obtained as white powder.

Table 1 shows Polymerisation conditions and yield and molecular weight characteristics of polymerisations conducted with macroinitiator 2 derived from PEG of molecular weight 2000 g/mol. In Table 1, [1] EC = ethylene carbonate

[2] Gel permeation chromatography used DMF eluent with PMMA standards

[3] The reaction mixture was purged with argon for 1 hour.

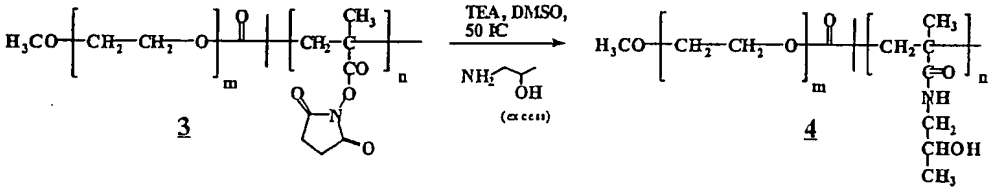
Table 1

	1 mmol (g)	2 (mmol) (g)	CuBr (mmol) (g)	Bpy (mmol) (g)	1:2:CuBr: bpy	EC[1] (g)	T (°C)	Time (h)	Yield (%)	Mn[2]	PD (Mw/Mn)
1	5 0.917	0.1 0.215	0.1 0.0157	0.2 0.0342	50:1:1:2	1.3	80	1.5	56	17,000	1.39
2	5 0.917	0.1 0.215	0.1 0.0157	0.2 0.0342	50:1:1:2	1.0	80	1.0	40	17,600	1.30
3	5 0.917	0.1 0.215	0.1 0.0157	0.2 0.0342	50:1:1:2	1.3	80	5.0	87	20,100	1.53
4	5 0.917	0.05 0.1075	0.05 0.0078	0.1 0.0171	100:1:1:2	0.65	80	5.0	100	31,300	1.32
5	2.5 0.458	0,05 0.1075	0.05 0.008	0.1 0.0171	50:1:1:2	0.65	60	5.0	20	13,230	1.35
6	5 0.917	0.1 0.215	0.1 0.0157	0.2 0.0342	50:1:1:2	1.3	80	5.5	32	19,700	1.29
7	2.5 0.458	0,05 0.1075	0.05 0.005	0.1 0.0171	50:1:1:2	0.65	100	5.0	64	28,000	1.38
8	2.5 0.458	0,5 0.1075	0.05 0.008	0.1 0.0171	50:1:1:2	0.65	80	5.0	56	25,700	1.29
9	2.5 0.458	0,05 0.1075	0.05 0.008	0.1 0.0171	50:1:1:2	0.65	80	5.0	47	22,800	1.27
10	2.5 0.458	0,05 0.1075	0.05 0.008	0.1 0.017	50:1:1:2	0.5	80	5.0	74[3]	30,200	1.28
11	2.5 0.458	0,05 0.1075	0.025 0.004	0.15 0.017	50:1:0.5:3	0.5	80	3.0	42.4[3]	18,100	1.25
12	5 0.917	0,05 0.1075	0.05 0.008	0.1 0.0171	100:1:1:2	0.5	110	4:35	100	18,600	1.33

Example 3

Conjugation reactions are included to demonstrate utility of the precursor to make functionalised polymers in narrow molecular weight distribution.

Conjugation reactions of polymer precursor 3.

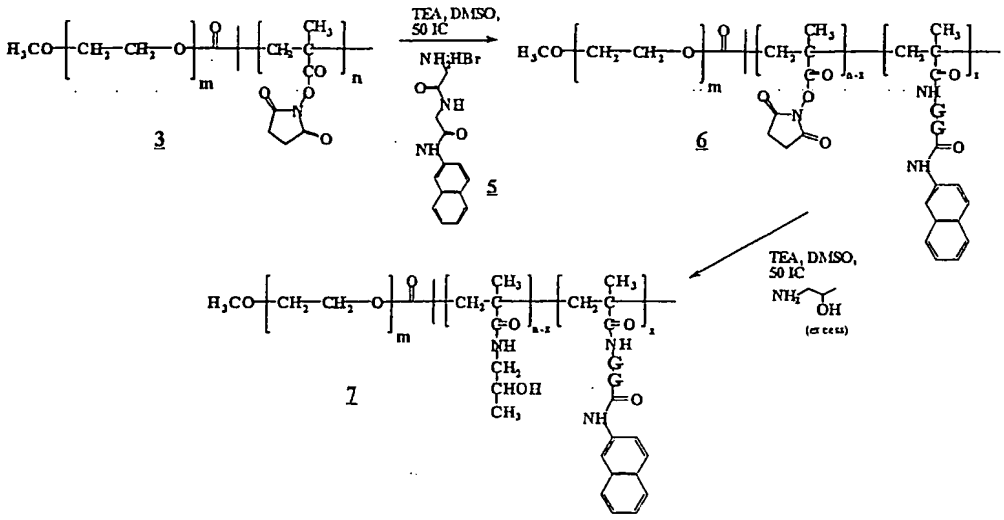


3 4

The co-blocked precursor 3 (0.1 g; Mn = 32,500 g/mol as determined by GPC with DMF eluent) was dissolved in anhydrous DMSO (0.3g) and purged with argon for 15 min. 1-Amino-2-propanol (0.2g) was dissolved in anhydrous DMSO (0.1 g) and purged with argon for 15 min, then the vial equipped with stirrer, was placed in an oil bath at 50 °C. The polymer solution was added dropwise (for ~15 min) by a syringe. The reaction mixture was allowed to react for 1.5 h. Then the product was precipitated in acetone:ether (1:1). The water soluble co-block polymer 4 was dissolved in MeOH and re-precipitated in acetone:ether =(1:1). The IR spectrum displayed no absorption at 1732 cm^{-1} indicating all the N-hydroxysuccinimide had been displaced. GPC analysis indicated the Mn was 25,500 g/mol (PD = 1.35) demonstrating that the hydrodynamic radius of polymer 4 differed significantly from the starting precursor polymer 3. H-NMR analysis of product 4 demonstrated the PEG block was covalently bound.

Example 4

Conjugation of 10 mole percent peptide drug model 5 followed by conjugation of 1-amino-2-propanol to give coblocked conjugate 7.



The co-blocked precursor 3 (0.15 g; Mn = 19,700 g/mol as determined by GPC with DMF eluent) was dissolved in anhydrous DMSO (1.25 ml) and purged with argon for 10 min. Since the macroinitiator 2 was found to have a GPC molecular weight Mn = 4,400 g/mol, the amount of reactive units in this sample of precursor polymer 3 was calculated to be 6.3×10^{-4} mol. To conjugate 10 mole percent 5, 6.3×10^{-5} mol (0.022g) of 5 was added to the reaction mixture and the flask placed in an oil bath at 50 °C. A solution of triethylamine (12.6×10^{-4} mol, 0.0127g) in 0.25ml DMSO (previously purged with Ar) was added drop-wise over 1-2 min. The mixture was stirred 10 min and then the solution of 1-amino-2-propanol (12.6×10^{-4} mol = 0.0946g) in 0.5 ml DMSO was added drop-wise for 10 min and the stirring continued for additional 50 min at 50 °C. The IR spectrum displayed no absorption at 1732 cm^{-1} indicating all the N-hydroxysuccinimide had been displaced and the conjugate was isolated in cold THF after centrifugation. H-NMR analysis of product 7 demonstrated the PEG block was covalently bound.